

Exposure–Response Associations of Household Air Pollution and Buccal Cell Telomere Length in Women Using Biomass Stoves

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BACKGROUND: Telomere shortening is associated with early mortality and chronic disease. Recent studies indicate that environmental exposures, including urban and traffic-related air pollution, may shorten telomeres. Associations between exposure to household air pollution from solid fuel stoves and telomere length have not been evaluated.

METHODS: Among 137 rural Chinese women using biomass stoves (mean = 55 y of age), we measured 48-h personal exposures to fine particulate matter [$PM \leq 2.5 \mu m$ in aerodynamic diameter ($PM_{2.5}$)] and black carbon and collected oral DNA on up to three occasions over a period of 2.5 y. Relative telomere length (RTL) was quantified using a modified real-time polymerase chain reaction protocol. Mixed effects regression models were used to investigate the exposure–response associations between household air pollution and RTL, adjusting for key sociodemographic, behavioral, and environmental covariates.

RESULTS: Women's daily exposures to air pollution ranged from 13–1,136 $\mu g/m^3$ for $PM_{2.5}$ (mean = 154) and 0.1–34 $\mu g/m^3$ for black carbon (mean = 3.6). Natural cubic spline models indicated a mostly linear association between increased exposure to air pollution and shorter RTL, except at very high concentrations where there were few observations. We thus modeled the linear associations with all observations, excluding the highest 3% and 5% of exposures. In covariate-adjusted models, an interquartile range (IQR) increase in exposure to black carbon (3.1 $\mu g/m^3$) was associated with shorter RTL [all observations: -0.27 (95% CI: $-0.48, -0.06$); excluding highest 5% exposures: -1.10 (95% CI: $-1.63, -0.57$)]. Further adjustment for outdoor temperature brought the estimates closer to zero [all observations: -0.15 (95% CI: $-0.36, 0.06$); excluding highest 5% exposures: -0.68 (95% CI: $-1.26, -0.10$)]. Models with $PM_{2.5}$ as the exposure metric followed a similar pattern.

CONCLUSION: Telomere shortening, which is a biomarker of biological aging and chronic disease, may be associated with exposure to air pollution in settings where household biomass stoves are commonly used. <https://doi.org/10.1289/EHP4041>

Introduction

Telomeres are repetitive nucleotide sequences that cap and protect the ends of chromosomes from degradation and inaccurate recombination (Blackburn 2001). Telomeres shorten with cell division owing to the inability of DNA polymerases to replicate ends of linear chromosomes (Bakaysa et al. 2007), which can eventually lead to cell senescence and apoptosis (Blackburn 2000). Experimental studies *in vitro* and in humans indicate that the rate of telomere shortening is accelerated by oxidative stress and inflammation responses (Aikata et al. 2000; von Zglinicki 2002), both of which are considered key determinants of biological aging (Correia-Melo et al. 2014) and contributors to the

development of chronic diseases including cancer and cardiovascular disease (Reuter et al. 2010; Siti et al. 2015).

Environmental factors, including exposures to tobacco smoke (Birch et al. 2015) and urban air pollution (Martens and Nawrot 2016), have been shown to induce cellular oxidative stress and systemic inflammation and may impact the rate of telomere length attrition by accelerating cell division or inducing DNA damage (Monaghan 2010). Long-term exposure to urban and traffic-related air pollution has been associated with shorter telomere length in a number of studies conducted in healthy adults and elderly populations (Miri et al. 2019). Shorter telomere length has been associated with greater risk of various aging-related health outcomes, including all-cause mortality (Cawthon et al. 2003; Mons et al. 2017), cancer (Zhu et al. 2016), hypertension (Tellechea and Pirola 2017), diabetes (Willeit et al. 2014), coronary artery disease (Haycock et al. 2014), and chronic obstructive pulmonary disease (Rode et al. 2013).

Household air pollution from stoves that burn biomass fuel (e.g., wood, crop residues, dung) is a pervasive environmental exposure impacting over 2.4 billion people globally (HEI 2018). It has been associated with adverse health outcomes throughout the life course, including low birth weight (Amegah et al. 2014), respiratory infections in young children (Smith et al. 2011), cancer (Mumford et al. 1987), and cardiopulmonary outcomes (Kurmi et al. 2010; Yu et al. 2018). Because air pollution plays a critical role in inducing oxidative stress and inflammation, we hypothesized that exposure to household air pollution from biomass

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stoves was inversely associated with telomere length. Our small ($n=21$) feasibility study found evidence of an inverse association between 24-h exposure to fine particulate matter [$PM \leq 2.5 \mu m$ in aerodynamic diameter ($PM_{2.5}$)] and buccal (cheek) cell telomere length in biomass stove users (Shan et al. 2014) but could not adjust for a number of important socioeconomic, dietary, and behavioral confounders. To our knowledge, no other studies have assessed the associations of household air pollution and telomere length, in large part due to the challenges of measuring exposure (Ezzati and Baumgartner 2017). To examine the exposure–response associations between air pollution from biomass stoves and relative telomere length (RTL), we conducted a panel study in a population of rural Chinese women who used wood-burning stoves in their homes.

Methods

Study Design and Location

In 2014, we enrolled 205 Chinese women 28–88 y of age into a longitudinal study of rural energy, air pollution, and health. Details about the study design and location are provided elsewhere (Ni et al. 2016; Shan et al. 2014). Briefly, our study area included 12 rural villages located in a mountainous region along the eastern edge of the Tibetan Plateau in Beichuan County, Sichuan, China. These villages were selected for the study because of a planned energy intervention program that included distribution of a multipurpose stove, chimney, and processed biomass fuel as part of a regional post-earthquake recovery program supported by China's Ministry of Science and Technology and the Ministry of Agriculture (Clark et al. 2017). Between May 2014 and February 2017, staff traveled to participants' homes to measure their personal exposure to air pollution and collect demographic, energy use, and health information during five data collection campaigns. Women who participated in the first winter data collection campaign of the longitudinal study were eligible for the present study ($n=174$), which involved the additional collection of oral DNA samples during three data collection campaigns, two in winter (2015 and 2016) and one in summer (2016).

Women were eligible for enrollment into the longitudinal study if they lived in villages where the energy intervention program was being implemented, reported cooking with traditional wood chimney stoves on most days, and were not pregnant. Enrollment was limited to women because they are the primary household stove users and because smoking prevalence among men was very high ($>60\%$), whereas none of the women reported smoking. Women interested in participation were introduced to the study by local staff and provided consent. Additional details on recruitment and enrollment are provided elsewhere (Ni et al. 2016). Study protocols were approved by ethical review boards at the University of Minnesota (no. 1304S31002), McGill University (no. A01-E01-14A), University of Wisconsin-Madison (no. 2014-0006), and Tsinghua University.

Household Stove and Fuel Use

All study homes used a traditional wood-burning stove for cooking, heating, and other household energy tasks, although many (42%) also used at least one clean fuel stove (e.g., gas, semi-gasifier, and/or electric induction stove). Wood stoves had enclosed combustion chambers and chimneys that vented smoke outdoors. Air pollutants were released into kitchens during lighting and refueling and from smoke that escaped the wood stove combustion chamber openings. Smoke from neighbors' wood stoves also impacted indoor air quality (Snider et al. 2018). Centralized heating was unavailable, so most homes burned wood or wood-charcoal in firepans or in their chimney stoves for heating. Detailed information on the energy use

practices of our study homes is published elsewhere (Clark et al. 2017; Shan et al. 2014).

Measurement of Personal Exposure to Air Pollution

We measured women's integrated personal exposures to $PM_{2.5}$ in the 48-h prior to DNA collection using lightweight, portable air samplers composed of small pumps (Apex Pro, Casella CEL) and personal exposure monitors (PEMs) (Harvard School of Public Health) with a mass median diameter of $2.5 \mu m$ when the pumps were operated at 1.8 Lpm ($\pm 10\%$) (Demokritou et al. 2001). The PEMs held 37-mm polytetrafluoroethylene (PTFE) filters (Zefluor, Pall Life Sciences) with $2.0\text{-}\mu m$ pore size and were secured to the outside of a waist pack with the inlet directed away from the participant's body. Participants were instructed to wear the waist pack at all times or keep them within 1–2 m while sitting, sleeping, or bathing. Compliance in wearing the monitors was evaluated through home visits and pedometers (HJ-321 Tri-Axis) placed inside the waist packs. Participants not wearing the monitors at the time of the home visits or who had pedometer counts of less than 1,000 steps were considered potentially noncompliant (9.4% of observations).

Pump flow rates were measured using a rotameter that was calibrated at the start of each field season using a primary gas flow standard (mini-BUCK Calibrator M-5, Buck Inc.). Prior to each sampling period, the PTFE filters were removed from numbered petri dishes and loaded into PEMs in a clean environment. The PEMs were placed into airtight, antistatic bags until they were attached to pumps in the participants' homes. At the end of each sampling period, staff placed the PEMs back into the antistatic bags and transported them back to the clean environment. The filters were removed from the PEMs, returned to their appropriate petri dishes, and stored in a freezer at $20^\circ C$. The PEMs were thoroughly wiped three times with isopropyl alcohol between uses. For quality control and to address potential contamination, field blank filters (equal to $\sim 10\%$ field blank filters) were placed in identical PEMs, subjected to the same field conditions, and analyzed using the same protocol as the sample filters.

Analysis of PTFE Filters for Mass and Black Carbon

Before and after air sampling, the PTFE filters were conditioned in a temperature- and humidity-controlled environment for at least 24 h and then weighed for mass on a microbalance (MX-5; Mettler-Toledo) at the Wisconsin State Laboratory of Hygiene. The balance's zero and span were checked after every batch of 10 filters. Each filter was weighed at least twice. If the first two weights differed by $>5 \mu g$ ($\sim 2\%$ of filters), the filter was reweighed until a stable weight was achieved. The average of the closest two weights was used for analysis.

Next, we analyzed the filters for black carbon, a combustion by-product that is more specific to biomass smoke than $PM_{2.5}$ (Bond et al. 2004), by measuring light attenuation by particles on the filter (SootScan™ OT21 Transmissometer, Magee Scientific). Black carbon measurements were calibrated using an empirical correction derived from co-located 48-h samples collected in a subsample of study homes ($n=57$) to measure black carbon and thermal-optical elemental carbon separately. Briefly, we applied spline regression models with 2, 3, and 4 degrees of freedom (df) to visualize the association between black carbon and elemental carbon. At higher concentrations, black carbon mass loading (in micrograms per centimeter squared) increased at a proportionally lower rate compared with measured elemental carbon, which was most apparent when black carbon mass loadings were $>35 \mu g/cm^2$. Based on visual evaluation of the data, we selected a spline model with one knot at black carbon mass = $35 \mu g/cm^2$ to fit the association between measured 48-h black carbon (in micrograms per

centimeter squared) and measured 48-h elemental carbon (in micrograms per centimeter squared). Measurements from different seasons were combined given that there was no seasonal difference in the association. We used the estimated black carbon–elemental carbon relationship from the spline model (R^2 value = 0.84) to predict corrected black carbon mass for all personal exposures. Corrected black carbon mass loading (in micrograms per centimeter squared) was converted to black carbon concentration by multiplying the mass loading (in micrograms per centimeter squared) by the area of each filter (9.03 cm^2) and then dividing that mass by the volume of air that was sampled during air pollution measurement (in cubic meters).

Field blanks yielded $\text{PM}_{2.5}$ mass and black carbon measurements that were higher than the instrument limits of detection reported by the manufacturers, and therefore detection limits were quantitated as three times the standard deviation (SD) of the field blanks ($\text{PM}_{2.5}$ ($n = 37$) = $6.3 \mu\text{g}$ and black carbon ($n = 21$) = $1.5 \mu\text{g}$). Additional information on PTFE filter analysis and related quality control procedures is provided elsewhere (Baumgartner et al. 2011; Shan et al. 2014).

Questionnaires and Other Measurements

Field staff administered short questionnaires to participants in Mandarin Chinese to obtain information on household demographics, energy use, and ventilation; socioeconomic status based on asset ownership [e.g., solar water heater, digital versatile disc (DVD) player, refrigerator, car, computer]; exposure to environmental tobacco smoke; self-reported health status; current medication use; and alcohol consumption. Questions were adapted from previous surveys conducted in rural China (Baumgartner et al. 2011) and retested prior to implementation. Women's height, weight, and waist circumference were measured at each visit without shoes and in lightweight clothing in their living rooms or bedrooms. Because most food and drink consumed by participants was prepared in their homes, we estimated individual dietary sodium intake based on 48-h household use of sodium-based seasonings and sauces divided by the number of household occupants. Physical activity (i.e., steps) was measured using a pedometer that was placed inside of the air monitoring waist pack worn by participants. Ambient air temperature was measured throughout the study at a centrally located meteorological station. Indoor temperature was measured at the time of health measurements and in the same room, and was nearly identical to outdoor temperature because centralized heating was unavailable and most space heating occurred in kitchens. Detailed information on these measurements is published elsewhere (Baumgartner et al. 2018).

Buccal Cell Collection

Participants were asked to refrain from eating for at least 2 h prior to buccal cell collection. Before collecting the sample, field staff asked participants to rinse their mouths twice with 100 mL of water for at least 15 s to remove any food particles. Staff also evaluated the participants for any oral lesions or wounds. Sterile nylon bristle brushes were rotated at least 10 revolutions against the inner part of the cheek in a circular motion to collect epithelial cells from the buccal mucosa. The left and right cheeks were sampled using separate toothbrushes to maximize DNA collection. Brush heads were placed inside 20-mL conical screw cap tubes containing 15 mL of fresh buffer solution and vigorously agitated to dislodge the cells into the buffer. After collection, the samples were placed in a cooler with blue ice and transported to a field laboratory within an hour. We stored the samples in a -30°C freezer until they were shipped on dry ice to Beijing for

DNA analysis. Details of this collection method are reported elsewhere (Shan et al. 2014).

Measurement of Relative Telomere Length

RTL was measured at the Beijing University of Chemical Technology in Beijing (China) using a modified quantitative real-time polymerase chain reaction (qRT-PCR) protocol, which is detailed elsewhere (Cawthon 2002). Briefly, this method quantifies RTL in oral DNA by determining the ratio of telomere repeat sequence copy number (T) to single-copy gene (S) ratio in individual samples and then compares them with a reference DNA sample. The reference sample in our study was generated by pooling samples from 10 randomly selected study participants and was used to create a standard curve (Cawthon 2002). Higher T:S ratios indicate longer telomeres.

Each sample was amplified for telomeric DNA and for beta-globin using the SYBR[®] Green method. The telomere qPCR primers were Tel1 5'-GGTTTTTGA[GGGTGA]4GGGT-3' and Tel2 5'-TCCCGACTAT[CCCTAT]4CCCTA-3', and the primers for beta-globin (human single-copy gene) were 5'-CAGCAAGTGGGAAGGTGTAATCC-3' and 5'-CCCATTCTATCATCAA CGGGTACAA-3'. Each PCR reaction mixture contained a 5-ng/ μL concentration of DNA and 1 \times SYBR Green Master Mix (TaKaRa). The thermal cycling conditions were 95°C for 30 s followed by 40 cycles of 95°C for 5 s, 54°C for 30 s, and 72°C for 31 s. PCR reactions were conducted on a QuantStudio 6 Flex Real-Time PCR system (Applied Biosystems[™]). Laboratory personnel were blinded to participants' characteristics and exposures, and all samples were processed in duplicate by the same technician and under identical conditions. The mean of two measurements was used for statistical analyses.

DNA concentration and purity were quantified by absorbance based on optical density (OD) at 260-nm wavelength (OD260) and the ratio of OD260 to OD at 280 nm wavelength (OD260:OD280). Based on the different DNA concentrations of each sample, different volumes were added into each well to ensure that the same amount of DNA was analyzed across samples. Quality control samples were interspersed throughout the plates in order to assess the interplate and intraplate variability. The interplate and intraplate coefficients of variation for duplicate measurements of cycle threshold (C_t ; number of cycles required for a fluorescent signal to detect a positive reaction in qRT-PCR) were less than 2%. The intraclass correlation coefficients for duplicate measurements of beta-globin (0.91) and telomeric DNA (0.98) were above 0.90, indicating excellent reliability (Koo and Li 2016).

Statistical analysis

We conducted univariate and multivariable mixed effects regression models with restricted maximum likelihood (REML) to investigate the associations between personal exposure to household air pollution and RTL (Laird and Ware 1982). Individual-level random effect estimates accounted for repeated measurements in more than one season. We conducted separate regression models for $\text{PM}_{2.5}$ and black carbon given their correlation (Spearman $r = 0.73$). Model assumptions of normality of random effects were verified using routine regression diagnostics (Lange and Ryan 1989).

For continuous variables, including air pollution, we assessed the response function for telomere length using natural cubic spline models with 2, 3, and 4 df. We selected the model through visual inspection of the response function and a comparison of the Akaike information criterion (AIC; a measure of goodness of fit), with a lower AIC suggesting a better fit to the data, although we excluded smoothers that produced implausible,

rapidly changing, nonmonotonic response patterns (Harrell 2001). We then computed the pointwise confidence intervals (CIs) and plotted the fitted functions using methods described by Cao et al. (2006). Response functions that were consistent with a linear association were replaced by linear functions.

Covariates included in the final multivariable models were either selected *a priori* as known confounders from the literature, or they were identified as potential confounders and their inclusion changed the estimate of PM_{2.5} or black carbon exposure by more than 5%. Factors selected *a priori* included age (Mather et al. 2011), socioeconomic status (Robertson et al. 2013), exposure to environmental tobacco smoke (Astuti et al. 2017), waist circumference (Wulaningsih et al. 2016), dietary sodium intake (Zhu et al. 2015), and physical activity (Mundstock et al. 2015). Variables evaluated as potential confounders included ethnicity (Han vs. Qiang), secondary occupation (categorized as none, housework, tenant farming, and nonfarm labor work), the time of day and day of the week of buccal cell collection, body mass index, current use of antihypertensive medication, and self-reported health status (categorized as excellent, good, fair, or poor). Outdoor temperature in the 48 h prior to buccal cell collection was also evaluated. Temperature has been associated with stove use and air pollution (Ni et al. 2016), and previous studies found that winter season and colder outdoor temperatures were

associated with shorter telomere length in adults (Rehkopf et al. 2014), although animal studies found no association (McLennan et al. 2018; Noreikiene et al. 2017).

Potential effect modification by age (<or≥median age of 53 y), body composition (<or≥median waist circumference of 75 cm), and exposure to environmental tobacco smoke (no/yes) was assessed by adding multiplicative interaction terms into the final statistical models because previous studies have suggested that these factors may modify the air pollution–telomere length associations (Lu et al. 2017; Muezzinler et al. 2013, 2014).

We conducted multiple sensitivity analyses. To relax the random effect distributional assumptions, we fitted fixed effects models that included the individual cluster information directly in the model as a predictor rather than as a random effect but exclude participants with one measurement (Croissant et al. 2017). We then compared the fixed effects models' results with those from mixed effects models, limited to participants with repeated measures. In a separate analysis, we conducted models for only the summer season, which had the least variability in outdoor temperature (SD = 1.9°C compared with SD = 8.0°C with both seasons included). We also evaluated whether our results changed after excluding women with diabetes (*n* = 4) or those who reported often consuming alcohol (*n* = 6) given that some studies have found strong associations between these

Table 1. Characteristics of study participants [*n* (%) or mean (SD) and median (IQR)].

Characteristic	<i>n</i> (%)	Mean (SD)	Median (IQR)
Age (y)	—	54.3 (12.2)	53.0 (19.0)
Ethnicity			
Han	111 (81)	—	—
Qiang	26 (19)	—	—
Secondary occupation ^a			
None	69 (50)	—	—
Housework	57 (42)	—	—
Tenant farming	7 (5)	—	—
Nonfarm labor work	4 (3)	—	—
Socioeconomic status based on assets ^b			
Owned 0–2 of 8	9 (7)	—	—
Owned 3–4 of 8	37 (27)	—	—
Owned 5–6 of 8	63 (46)	—	—
Owned 7–8 of 8	28 (20)	—	—
BMI (kg/m ²)	—	25.0 (3.7)	24.5 (4.6)
Waist circumference (cm)	—	83.9 (10.0)	84.0 (14.0)
Lives with at least one tobacco smoker			
Yes	48 (35)	—	—
No	89 (65)	—	—
Alcohol consumption			
Daily	6 (4)	—	—
Occasionally	26 (19)	—	—
Never	105 (77)	—	—
Diabetes (self-reported)			
Yes	4 (3)	—	—
No	133 (97)	—	—
Dietary sodium intake (mg/person/day)	—	7,670 (6,964)	5,521 (6,858)
Physical activity [daily steps (<i>n</i>)]			
Summer	—	6,204 (7,331)	3,559 (5,583)
Winter ^c	—	6,140 (7,870)	3,975 (5,255)
PM _{2.5} exposure (μg/m ³)			
Summer	—	69.9 (57.6)	50.8 (38.5)
Winter ^c	—	220.8 (209.4)	151.7 (190.2)
Black carbon exposure (μg/m ³)			
Summer	—	2.1 (3.0)	1.3 (1.6)
Winter ^c	—	4.8 (5.7)	3.3 (3.1)
Relative telomere length			
Summer	—	10.3 (2.4)	10.3 (2.7)
Winter ^c	—	8.5 (1.9)	8.0 (2.1)

Note: Characteristics of the full study population (*n* = 205) can be found in Table S1. —, not applicable; BMI, body mass index; IQR, interquartile range; PM_{2.5}, particulate matter ≤ 2.5 μm in aerodynamic diameter; SD, standard deviation.

^aAll participants farmed their own land as a primary occupation.

^bAssets included a solar water heater, DVD player, refrigerator, computer, microwave, electric water heater/cooler, electric hotplate, and car.

^cFor participants with two winter season measurements (*n* = 16), we used the average of the two so that each participant only contributed one measurement per season.

factors and telomere length (Wang et al. 2016), although others did not (Weischer et al. 2014; You et al. 2012). Finally, we reconducted the analysis after excluding 18 observations where air pollution exposures did not meet the criteria $\pm 20\%$ of the target sampling time of 24, 48, or 72 h (i.e., a daily measure).

All statistical analyses were conducted in R (version 3.4.2; R Development Core Team), using the “nlme” package for mixed models and “splines” for natural cubic splines.

Results

We enrolled 137 participants of 175 eligible women into this study, upon agreement to have their oral DNA collected (79% participation rate) (Table 1). Of these, 78 participated in one season and 59 in two or more seasons between November 2013 and December 2015. Just 8 women participated in three data collection campaigns because the duration of one winter campaign was shortened due to field logistics and a national holiday. Reasons for not participating in more than one campaign included temporary relocation to care for grandchildren (30%), toothache or mouth sores that prevented buccal cell collection (25%), insufficient cell collection supplies on the measurement day (29%), refusal (15%), or death (1%). DNA was successfully extracted from 198 of 207 buccal cell samples (96%) collected during the study.

Women’s mean \pm SD personal exposures to PM_{2.5} were two to six times higher than the World Health Organization’s interim indoor air quality target of 35 $\mu\text{g}/\text{m}^3$ [winter ($n=95$): $229.8 \pm 218.0 \mu\text{g}/\text{m}^3$; summer ($n=87$): $69.7 \pm 58.0 \mu\text{g}/\text{m}^3$]. Exposures to black carbon followed a similar pattern [winter ($n=95$): $4.9 \pm 5.7 \mu\text{g}/\text{m}^3$; summer ($n=86$): $2.1 \pm 3.0 \mu\text{g}/\text{m}^3$] (Table 1). The ranges of exposure were 13–1,136 $\mu\text{g}/\text{m}^3$ for PM_{2.5} and 0.1–34 $\mu\text{g}/\text{m}^3$ for black carbon. Based on the intra-class correlation coefficient (ICC=0.15), we observed high within-individual variability relative to total variability in PM_{2.5} exposure. Average telomere length was shorter in winter ($n=99$; mean = 8.5 ± 2.0) than in summer ($n=84$; mean = 10.3 ± 2.4), and varied by level of exposure to air pollution (Figure 1).

Assessment of Response Functions

The associations between RTL and age, socioeconomic status, waist circumference, sodium intake, and daily steps were consistent with linearity. Figure 2 shows the associations between RTL and air pollution using 2 df natural cubic spline, including the univariate and adjusted models. Although at first glance there

appears to be a nonlinear pattern where the association flattens or slightly increases at very high levels of exposure, the nonlinearity for higher concentrations is extremely uncertain as evidenced by the few observations and hence the very wide confidence bands. We thus concluded that the relationships were overall consistent with linearity, and therefore we report the change in RTL and 95% CIs associated with 10- $\mu\text{g}/\text{m}^3$ and 1- $\mu\text{g}/\text{m}^3$ increases in exposure to PM_{2.5} and black carbon, respectively, for all observations and then excluding observations with the highest 3% and 5% of exposures. To facilitate comparison between pollutant models, we also present the change in RTL associated with an interquartile range (IQR) increase in exposure to air pollution (151.3 $\mu\text{g}/\text{m}^3$ for PM_{2.5} and 3.1 $\mu\text{g}/\text{m}^3$ for black carbon).

Associations between Air Pollution and Relative Telomere Length

In the univariate and multivariable mixed-effect models, increased exposures to both PM_{2.5} and black carbon were inversely associated with RTL (Table 2). In multivariable models including all observations, a 1- $\mu\text{g}/\text{m}^3$ increase in black carbon was associated with -0.09 (95% CI: $-0.16, -0.02$) shorter telomere length. Adjusting for ambient temperature further reduced the association (-0.05 ; 95% CI: $-0.09, -0.02$). As expected based on the spline regression models, removing the highest 3% and 5% of exposure observations resulted in 49–354% larger associations. Models with PM_{2.5} exposure followed similar trends. An IQR increase in black carbon was associated with decreases in RTL that were of a similar magnitude to PM_{2.5} in the multivariable models [black carbon: -0.27 (95% CI: $-0.48, -0.06$) vs. PM_{2.5}: -0.40 (95% CI: $-0.69, -0.12$)] and additionally adjusting for temperature reduced the association [black carbon: -0.15 (95% CI: $-0.36, -0.06$) vs. PM_{2.5}: -0.09 (95% CI: $-0.41, 0.22$)].

The fixed effects models followed similar trends to the mixed effects models with all participants included and when limited to participants with repeated measures (Table 3). In summer season-specific models, the air pollution–telomere associations were slightly larger than in models with both seasons included, likely because the higher wintertime exposures were excluded, and temperature was no longer a confounder (see Table S2). The associations between air pollution and telomere length were not modified by age, body composition, or exposure to environmental tobacco smoke (all interaction term $p > 0.18$; see Table S3). Excluding women with diabetes ($n=4$) and those who reported often

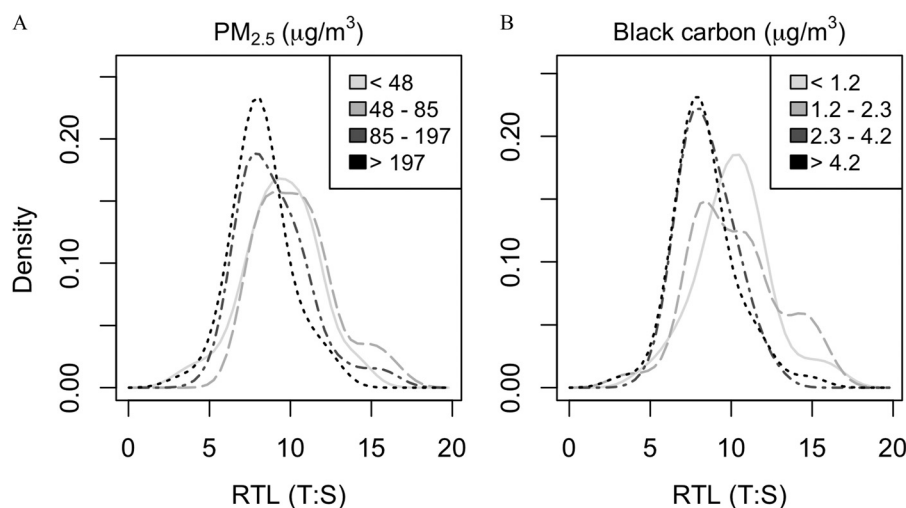


Figure 1. Distribution of relative telomere length (T:S) by quartile of exposure to air pollution for (A) PM_{2.5} and (B) black carbon. PM_{2.5}, particulate matter $\leq 2.5 \mu\text{m}$ in aerodynamic diameter; T:S, telomere repeat sequence copy number to single-copy gene ratio.

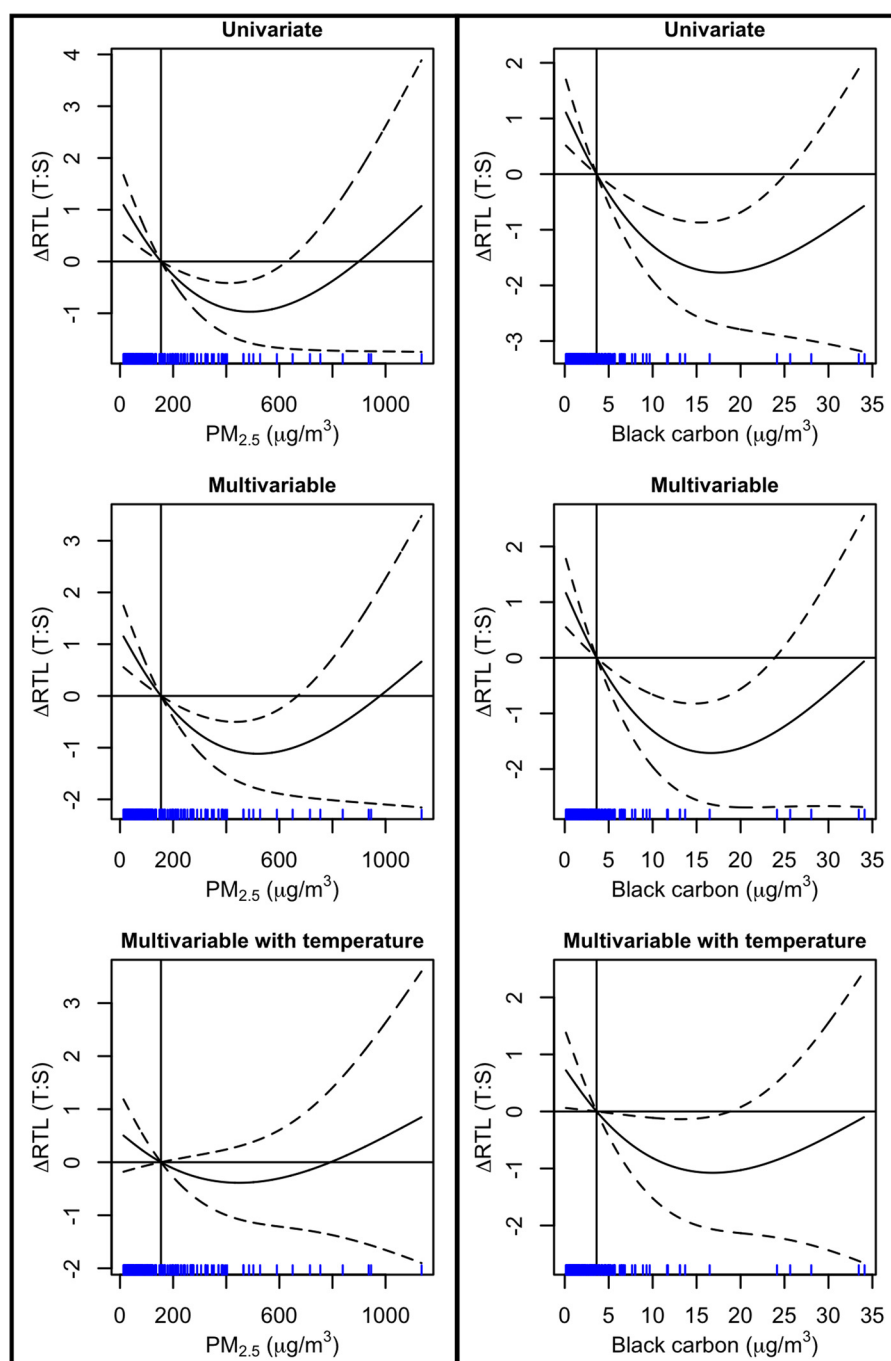


Figure 2. Natural cubic splines with 2 degrees of freedom (solid line) and associated 95% confidence intervals (dashed lines) illustrating the associations between relative telomere length (T:S) and exposures to $\text{PM}_{2.5}$ and black carbon ($\mu\text{g}/\text{m}^3$) among rural Chinese women using household biomass stoves. Mean change is relative to the mean exposure (vertical black line). Results presented as univariate (top); multivariable models adjusted for age, waist circumference, socioeconomic status, exposure to environmental tobacco smoke, dietary sodium intake, physical activity, and the time of day and day of the week of DNA collection (middle); and ambient temperature (bottom). The vertical cyan lines along the x-axes show the distribution of the pollutant exposures. $\text{PM}_{2.5}$, particulate matter $\leq 2.5 \mu\text{m}$ in aerodynamic diameter; T:S, telomere repeat sequence copy number to single-copy gene ratio.

consuming alcohol ($n = 6$) did not appreciably change our results (data not shown). Finally, excluding 18 (9%) observations where the duration of air pollution measurement failed to meet the daily target also did not change our results (see Table S4).

Discussion

In our analysis of 137 rural Chinese women using household biomass stoves, we found that increased personal exposures to

$\text{PM}_{2.5}$ and black carbon were associated with shorter buccal cell telomere length. This study is the first exposure–response study of household air pollution and telomere length. Air pollution from biomass stoves is one of the world’s most pervasive environmental exposures, impacting the indoor air quality of homes using the stoves and the outdoor air quality of the local community and surrounding region (Carter et al. 2016; Chafe et al. 2014). This study adds to existing evidence indicating the adverse health impacts of household biomass burning given that telomeres play an important

Table 2. Associations between relative telomere length (T:S) and personal exposures to air pollution in rural Chinese women using household biomass stoves. Results from mixed effects models.

			Difference in relative telomere length (95% CI) ^b		
Exposure	Women (n) ^a	Obs (n)	Univariate	Multivariable ^c	Multivariable ^c plus temperature
PM _{2.5} (per 10 µg/m ³)					
All exposures	132	195	−0.02 (−0.04, −0.01)	−0.03 (−0.05, −0.01)	−0.01 (−0.03, 0.01)
<601 µg/m ³	128	189	−0.05 (−0.08, −0.02)	−0.06 (−0.08, −0.03)	−0.03 (−0.06, 0.01)
<472 µg/m ³	128	185	−0.06 (−0.09, −0.03)	−0.06 (−0.09, −0.03)	−0.03 (−0.06, 0.01)
PM _{2.5} (per IQR)					
All exposures	132	195	−0.36 (−0.64, −0.09)	−0.40 (−0.69, −0.12)	−0.09 (−0.41, 0.22)
<601 µg/m ³	128	189	−0.79 (−1.21, −0.38)	−0.83 (−1.26, −0.41)	−0.41 (−0.89, 0.08)
<472 µg/m ³	128	185	−0.88 (−1.36, −0.41)	−0.90 (−1.40, −0.41)	−0.39 (−0.96, 0.17)
Black carbon (per 1 µg/m ³)					
All exposures	131	194	−0.10 (−0.17, −0.03)	−0.09 (−0.16, −0.02)	−0.05 (−0.12, 0.02)
<13.9 µg/m ³	127	188	−0.21 (−0.35, −0.07)	−0.21 (−0.36, −0.07)	−0.09 (−0.24, 0.07)
<10.5 µg/m ³	125	184	−0.35 (−0.51, −0.18)	−0.35 (−0.53, −0.18)	−0.22 (−0.41, −0.03)
Black carbon (per IQR)					
All exposures	131	194	−0.31 (−0.51, −0.10)	−0.27 (−0.48, −0.06)	−0.15 (−0.36, 0.06)
<13.9 µg/m ³	127	188	−0.65 (−1.08, −0.22)	−0.66 (−1.11, −0.21)	−0.27 (−0.75, 0.20)
<10.5 µg/m ³	125	184	−1.07 (−1.59, −0.56)	−1.10 (−1.63, −0.57)	−0.68 (−1.26, −0.10)

Note: CI, confidence interval; IQR, interquartile range; obs, observations; PM_{2.5}, particulate matter ≤2.5 µm in aerodynamic diameter; T:S, telomere repeat sequence copy number to single-copy gene ratio.

^aN < 137 women due to unsuccessful extraction of DNA (n = 3) or missing air pollution measurements due to filter damage or instrument error (PM_{2.5} = 2, black carbon = 3).

^bDifference in relative telomere length (T:S) associated with a 10-µg/m³ increase in exposure to PM_{2.5} or 1-µg/m³ increase black carbon with all observations and after excluding the highest 3% and 5% exposures, or with an IQR increase in exposure after excluding the highest 5% of exposures. IQRs for PM_{2.5} and black carbon were 151.3 and 3.1 µg/m³, respectively.

^cModel adjusted for age, waist circumference, socioeconomic status, exposure to environmental tobacco smoke, dietary sodium intake, physical activity, and the time of day and day of the week of DNA collection.

role in the maintenance of chromosome integrity and stability and the increasing evidence that shortened telomere length is associated with increased risk of chronic disease and early mortality (Haycock et al. 2014).

Women in our study were exposed to a wide range of daily levels of air pollution, ranging from 13–1,136 µg/m³ for PM_{2.5} and 0.1–34 µg/m³ for black carbon, and with large seasonal differences due to the contribution of winter space heating. Their exposure levels generally exceeded those typical of urban areas (HEI 2018), including high-pollution cities in China (Chen et al. 2017), but closely aligned with the levels of exposure commonly observed in settings where biomass stoves are an important source of air pollution (Carter et al. 2017). At our study site, biomass combustion was the largest contributor to both indoor and outdoor PM_{2.5}, and there was little variability in the composition and source contributors to indoor versus outdoor PM_{2.5}, likely

due to high rates of exchange between indoor and outdoor air (Lai et al. 2019). Exposures to PM_{2.5} and black carbon were highly correlated and their subsequent associations with telomere length were similar, likely because biomass burning is the major source of air pollution in our study setting. Average RTL also varied seasonally and was shorter in the winter compared with summer. This may be partly attributable to seasonality (Rehkopf et al. 2014) and the environmental and behavioral risk factors that also varied seasonally, including air pollution, temperature, and level of activity (Hou et al. 2012; Ni et al. 2016; Shan et al. 2014).

All of the air pollution–RTL associations were larger after removing the very highest exposures owing to some nonlinearity in the exposure–response relationship, as illustrated by our spline models. It is unlikely that air pollution has a protective effect on telomere length at very high concentrations, although previous

Table 3. Associations between relative telomere length (T:S) and personal exposures to air pollution in rural Chinese women using household biomass stoves. Results from the fixed effects (FE) models and mixed effects (ME) models limited to participants with repeated measurements.

Exposure	Model	Women (n)	Obs (n)	Difference in relative telomere length (95% CI) ^a		
				Univariate	Multivariable ^b	Multivariable ^b plus temperature
PM _{2.5} (per 10 µg/m ³)						
All exposures	FE	54	117	−0.05 (−0.09, −0.01)	−0.05 (−0.09, −0.01)	−0.01 (−0.06, 0.04)
	ME	54	117	−0.02 (−0.05, 0.003)	−0.02 (−0.05, 0.01)	0.004 (−0.03, 0.03)
<515 µg/m ³	FE	50	109	−0.12 (−0.17, −0.07)	−0.12 (−0.17, −0.06)	−0.08 (−0.15, −0.02)
	ME	50	109	−0.06 (−0.10, −0.02)	−0.06 (−0.1, −0.02)	−0.03 (−0.07, 0.02)
<469 µg/m ³	FE	48	105	−0.14 (−0.20, −0.08)	−0.14 (−0.20, −0.08)	−0.10 (−0.18, −0.03)
	ME	48	105	−0.06 (−0.1, −0.02)	−0.06 (−0.1, −0.01)	−0.02 (−0.07, 0.04)
Black carbon (per 1 µg/m ³)						
All exposures	FE	53	116	−0.07 (−0.24, 0.10)	−0.07 (−0.25, 0.11)	0.03 (−0.14, 0.21)
	ME	53	116	−0.06 (−0.17, 0.04)	−0.02 (−0.13, 0.09)	0.03 (−0.08, 0.14)
<10.7 µg/m ³	FE	49	108	−0.60 (−0.97, −0.23)	−0.57 (−1.00, −0.15)	−0.24 (−0.76, 0.28)
	ME	49	108	−0.38 (−0.61, −0.15)	−0.35 (−0.61, −0.1)	−0.21 (−0.48, 0.08)
<8.3 µg/m ³	FE	47	103	−0.75 (−1.11, −0.38)	−0.70 (−1.11, −0.29)	−0.41 (−0.91, 0.09)
	ME	47	103	−0.57 (−0.84, −0.31)	−0.58 (−0.86, −0.29)	−0.42 (−0.75, −0.09)

Note: CI, confidence interval; IQR, interquartile range; obs, observations; PM_{2.5}, particulate matter ≤2.5 µm in aerodynamic diameter; T:S, telomere repeat sequence copy number to single-copy gene ratio.

^aDifference in RTL associated with a 10-µg/m³ increase in exposure to PM_{2.5} or 1-µg/m³ increase in black carbon with all observations and after excluding the highest 3% and 5% of exposures. Results for models with IQR exposures are reported in Table S5.

^bModels adjusted for dietary sodium intake, physical activity, and the time of day and day of the week of DNA collection.

exposure–response studies of household air pollution and vascular outcomes do indicate that associations are steep at lower levels of exposure and flatten out at higher levels (Baumgartner et al. 2018). Rather, it is more likely that the very high exposures observed for a small number of our participants were not usual (i.e., they were a result of unusually long cooking or heating) or, despite extensive quality control procedures, were attributable to an unidentified error in the participant's PM measurement.

Adjusting for outdoor temperature decreased the coefficients for air pollution by 45–80%, making it the strongest confounder in our statistical models with both seasons included. The confounding influence of temperature was removed in the summer season–specific models when outdoor temperature varied only minimally, although the air pollution–telomere length associations remained. Our study homes used their biomass stoves more frequently and for longer periods when outdoor temperatures were colder, resulting in higher levels of air pollution (Carter et al. 2016). It is therefore very likely that some of the air pollution effect in our statistical models with both seasons was captured by temperature because these two variables are correlated (Spearman $r=0.43$ – 0.60) and temperature can usually be measured with greater precision. Further, an association between outdoor temperature and telomere length is not as well established as for other covariates included in our statistical models (Mather et al. 2011; Weischer et al. 2014; Wulaningsih et al. 2016).

Exposure to household air pollution may influence telomere length by increasing the replication rate of cells and by enhancing the extent of telomere loss during each replication. Fine PM emitted from biomass burning has high intrinsic oxidative potential and inflammatory properties (Liu et al. 2014; Secrest et al. 2016), which have been shown to accelerate telomere shortening in animal and cellular models (Jurk et al. 2014; Reichert and Stier 2017; Saretzki and von Zglinicki 2002). Oxidative stress is generated by an imbalance of reactive oxygen species (ROS) and antioxidants in the body and can be mediated by exposure to PM (Martens and Nawrot 2016) through a pathway that induces DNA damage by activating the intracellular production of ROS in inflammatory cells, thus creating a positive feedback loop that leads to chronic inflammation (Møller et al. 2014; Risom et al. 2005). Chronic inflammation, in turn, has been shown to increase the rate of telomere shortening and cellular senescence (Jose et al. 2017).

Our finding of an inverse association between household air pollution and RTL is consistent with our previous feasibility study on this topic where women exposed to higher levels of air pollution ($>58 \mu\text{g}/\text{m}^3$ over 24 h) had a 43% shorter buccal cell telomere length than women exposed to lower levels but was limited by a small sample size of 21 women (Shan et al. 2014). A recent cross-sectional study in northern Chinese adults found that self-reported use of coal stoves for three or more decades was associated with shorter blood leukocyte telomere length compared with adults using clean fuel stoves, although they did not adjust for socioeconomic variables (i.e., income, occupation, or education) even though socioeconomic variables were correlated with both fuel type and telomere length (Lin et al. 2017).

Our findings also support the relatively small number of studies on long-term exposure to air pollution and telomere length conducted in occupational and urban settings. Four panel studies found that exposures to PM_{10} over months or years was associated with shorter telomeres in Chinese office workers and truck drivers (Hou et al. 2012), elderly men in the United States (McCracken et al. 2010), an occupational cohort of boilermakers (Wong et al. 2014), and newborns in China (Perera et al. 2018). We consider our short-term (48-h) exposure measurement to be representative of longer-term usual exposure for two reasons. First, household biomass stoves are the primary source of

exposure to $\text{PM}_{2.5}$ in this population (Lai et al. 2019). Cooking and, in winter, heating with biomass stoves are daily activities and our study participants have used household biomass stoves throughout their lifetimes. The intensity and frequency of their stove use patterns can vary from day to day and seasonally (Carter et al. 2016), which we tried to capture by measuring exposure for 2 d in each seasonal campaign. Second, sensor-based measurements of stove use in our study showed good correlations between 48-h and longer-term (monthly and within-season) average daily stove use minutes (range of Pearson $r=0.55$ – 0.80) (Clark et al. 2017). These results support a longitudinal study in Guatemala that found that 48-h measurement of indoor $\text{PM}_{2.5}$ was predictive of the long-term daily average indoor $\text{PM}_{2.5}$ in homes where biomass stoves were a primary source of air pollution (Pillarisetti 2016).

In contrast to our study, acute exposures to PM in the 1–7 d prior to DNA collection were associated with rapid and significant increases in telomeres in adult workers and in children (Walton et al. 2016). The differential effects observed for acute versus long-term exposures may reflect the balance between the acute effects of inflammation and the long-term effects of oxidative stress. Experimental studies have shown that acute exposures to PM are associated with alveolar inflammation, either directly or via oxidative stress (Møller et al. 2014). *In vitro* studies have found increased expression of telomerase, a telomere-synthesizing enzyme, in B cells in the presence of inflammatory markers that was also correlated with a transient (several-day) increase in telomere length (Weng et al. 1997). Longer telomeres respond to inflammatory signals, which is thought to contribute to the proliferative capacity and clonal expansion necessary to generate an efficient inflammatory response (Hodes et al. 2002). In contrast, the cumulative burden of oxidative stress and inflammation from longer-term exposure to air pollution has been shown to decrease telomere length (Martens and Nawrot 2016). Together, these studies suggest that acute PM exposures might increase telomere length in the hours to days following exposure, which may subsequently sustain the inflammatory mechanisms associated with telomere shortening and PM health effects. Two studies with both short- (hours to several days) and longer-term (weeks to year) exposure assessment observed that short-term exposure to $\text{PM}_{2.5}$ was associated with increased telomere length, whereas long-term exposure was associated with shorter telomere length (Hou et al. 2012; Pieters et al. 2016). Longitudinal studies of household air pollution would be valuable for assessing the effects of acute versus longer-term exposures, although we acknowledge that long-term exposure assessment would be challenging.

We limited our analysis to telomeric DNA from buccal cells, which have the advantages of being noninvasive and relatively easy to collect and transport in remote settings such as ours. Buccal cells live in a highly oxygenated microenvironment that may be more vulnerable to change from oxidative stress and rates of cell division (Houben et al. 2008). It is not clear whether the rate of telomere shortening in buccal cells is reflective of telomere shortening in other tissues (Daniali et al. 2013), although strong intra-individual correlations between telomere length measured in buccal cell, blood, and fibroblast DNA using the same PCR method as used in our study have been observed (Finnicum et al. 2017; Gadalla et al. 2010). Leukocyte telomere length has been most commonly measured in urban air pollution studies (Miri et al. 2019). Notably, leukocytes have cell subpopulations with higher rates of telomere shortening and different replicative activity; thus, leukocyte telomere length may not be considered a good proxy for telomere length in other tissues (Sanders and Newman 2013). Future studies could evaluate the rates of telomere shortening in different tissues in response to exposure to air pollution.

Our study has several notable strengths. First, our repeated seasonal measurements of 48-h exposure to air pollution captured both day-to-day and seasonal variability and enabled us to estimate exposure–response relationships. Further, we were able to measure exposures to both PM_{2.5} and black carbon, a pollutant marker that is more specific to biomass smoke than PM_{2.5}. Second, we statistically controlled for a comprehensive set of sociodemographic, behavioral, and environmental variables that have been associated with telomere length in other settings and that were not adjusted for in the previous studies of biomass and coal stove users. Finally, although sample size of 137 women was relatively small in order to accommodate the personal exposure assessment and important confounding variables, the use of repeated measures in 42% of participants increased the statistical power of our study beyond its sample size (Weichenthal et al. 2017).

Several limitations should also be noted. Our study is limited by its cross-sectional design, although it is unlikely that small changes in telomere length would affect exposure to air pollution. It is possible that poor health status or advanced disease, which may be associated with shorter telomere length, could impact mobility and thus increase exposure to air pollution inside homes. However, our study population was overall quite healthy (Baumgartner et al. 2018) and adjusting for self-reported health status did not affect our results. Our 48-h exposure measurement was designed as a surrogate of longer-term exposure. Some degree of nondifferential measurement error in exposure assessment is expected, which should bias our results toward the null. Related to this, we also could not assess the time-dependent components of air pollution-induced changes in telomere length, which could be a subject of future longitudinal and intervention studies. This ancillary study was conducted in a nonrandom subsample of participants and selection bias is thus a possibility, although the sociodemographic characteristics and air pollution exposures of our subsample were very similar to the full study population. Finally, we cannot exclude the possibility of bias due to residual confounding. The most important potential confounders were assessed by trained staff using extensively field-tested questionnaires (i.e., age, asset-based socioeconomic status, exposure to environmental tobacco smoke), standardized procedures (i.e., waist circumference, weight, height), and objective measures (i.e., pedometer-based physical activity) and were statistically controlled for in all analyses. We were unable to assess lipids, psychosocial stress, and dietary factors beyond sodium, which have been previously associated with shorter telomeres in studies of adults in high-income countries (Lakowa et al. 2015; Rafie et al. 2017; Woody et al. 2017). However, it is unlikely that these unmeasured variables were associated with women's exposure to household air pollution in our study setting where all participants used wood-burning stoves.

Conclusions

Our study is the first to investigate the associations of exposure to household air pollution and RTL. These findings contribute to knowledge on the health impacts of air pollution at the molecular level and provide evidence that personal exposures to PM_{2.5} and black carbon from biomass stoves are associated with shortened telomere length in buccal cells among never-smoking women after adjusting for a comprehensive set of confounders. Our results reinforce the importance of reducing exposures to household air pollution.

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